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Development of a Model of Toluene
Blood Level Following Subcutaneous
Injection of Toluene in the Rat

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DEVELOPMENT OF A MODEL OF TOLUENE BLOOD LEVEL
FOLLOWING SUBCUTANEOUS INJECTION OF TOLUENE IN THE RAT

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The complexities of environmental problems originate in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines. The Health Effects Research Laboratory, Research Triangle Park, NC and Cincinnati, OH conducts a coordinated environmental health research program in toxicology, epidemiology and clinical studies using human volunteer subjects. Wide ranges of pollutants known or suspected to cause health problems are studied. The research focuses on air pollutants, water pollutants, toxic substances, hazardous wastes, pesticides, and non-ionizing radiation. The laboratory participates in the development and revision of air and water quality criteria and health assessment documents on pollutants for which regulatory actions are being considered. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of environmental regulatory decisions involving the protection of the health and welfare of all U.S. inhabitants.

This report addresses the issue of the development of a model for prediction of blood toluene level after subcutaneous injection of toluene. This methodological report will facilitate exposures to toluene so as to study its toxicological effects.

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PREFACE

The purpose of publishing these data as an internal publication rather than as a peer-reviewed journal article is to make available for public record the exact steps taken in developing this model. There were many decisions and choices en-route to the final model. It is important that the exact process be a matter of record.

An abbreviated version of this publication will be submitted to a peer-reviewed journal. In this abbreviated version, the methods section will contain a brief statement of the principles which guided the development of the model but all intermediate results will only be summarized. That article will concentrate upon the validation of the model.

ABSTRACT

A model of toluene level in blood following subcutaneous injection of toluene mixed with polyoxyethylated vegetable oil vehicle was developed. The purpose was to provide a means of predicting dose received, for subsequent toxicologic studies for any time and dose combination. The pharmacokinetics were of secondary interest. Using data from 111 rats, a 4-parameter equation was devised to predict the time course of toluene blood level from 20-480 min., for dose levels of 50-1000 mg/kg. Blood concentrations rose at a rate which was independent of dose level. Maximum blood levels were uniquely determined by dose level. Blood levels fell at different rates, depending upon dose level. Injection exposure, when compared to inhalation, has the advantages of (a) low expense (b) low equipment requirements and (c) simplicity. The disadvantage is, for some experiments, poor temporal simulation of the normal route administration, inhalation.

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SECTION 1

INTRODUCTION

Toluene is a commonly occurring substance in the modern environment. It is widely used as a solvent in paints, glue and rotogravure printing to name but a few applications (Benignus, 1981). Thresholds for effects on neural and behavioral functions range from concentrations of 100 ppm in ambient air to about 1000 ppm depending upon the dependent variables (Benignus 1981).

Humans are normally exposed to toluene via inhalation. Injection has been used by many investigators to provide a convenient and inexpensive alternative to the more complex technology of inhalation exposure. Despite the common use of this method, only one study was found in which tissue levels of toluene after injection were reported (Sato and Nakajima, 1979). Using intraperitoneal (IP) injections of 30, 115 and 460 mg/kg, they measured blood levels at 2, 4, 8, 12 and 24 hours in rats. Although most of the rising limb of the curve occurred before the first blood sample was taken, it appeared that toluene reached its peak blood value later for larger doses. Since statistical tests were not performed, it was not clear whether the elimination rate was a function of dose level. Sampling rate for blood level was also quite low. Due to infrequent and late sampling and lack of statistical testing of the curve fits, it is unclear whether the curves which they used adequately described the time course of blood levels.

Toluene has been injected both IP and subcutaneously (SC) by various investigators but blood levels over time were not reported. For many experiments it is desirable to have blood toluene levels maintained at a stable level for a long period of time rather than reach a peak and be eliminated rapidly. It is known that drugs delivered by SC injections are more slowly and evenly absorbed over time than are those given by IP injection (Goodman and Gillman, 1956). No data were found, however, on toluene blood levels after SC injections.

This study was performed in an effort to discover and describe the time course of toluene in blood after injection. Design of subsequent toxicologic studies would be greatly simplified with such information available. The pharmacokinetics were of secondary interest. SC injection was used because a long, slow absorption is often desirable. If the time course of toluene blood level after injection were known, at least preliminary studies could be done using injections rather than the more expensive and complex procedure of inhalation exposure.

SECTION 2

CONCLUSIONS

The time course of toluene in blood following SC injections was observed (see Figure 10). Rise rates, maximum blood level, and decline toluene in blood were all functions of dose level. A 4-parameter equation was devised to predict blood level as a function of time after injection and dose level. The purpose was to be able to predict dose received in subsequent toxicologic research, rather than investigate the pharmacokinetics.

The SC injection technique appears to have utility as a method of toluene exposure. It has the advantage of low expense and equipment requirements as well as simplicity of use. Its disadvantage reduces to poor temporal simulation of inhalation exposure for certain kinds of studies. Perhaps it would be most useful for preliminary work on the effects of toluene. Effects would have to be verified with inhalation exposures in order to generalize the results to the usual route of exposure, inhalation.

SECTION 3

MATERIALS AND METHODS

Experimental Design

The work in this report was an alternating series of exploratory and confirmatory studies. The process of devising the injection technique and the model for predicting results will be described in context in the Results section.

Animals

All 254 subjects were Long-Evans hooded rats of similar age. Table 1 shows mean ages and weights for the rats used in each of the 5 studies. All rats were kept in an animal colony on a night-dark schedule for a minimum of 30 days before use.

Injections

Injections were administered SC (subcutaneously) by grasping the rat with the non-dextrous hand and pushing the skin of the back upward to form a "hump". A 22 gauge, one inch needle on a plastic syringe was inserted into the skin hump, in an anterior direction parallel to the back of the rat. After the injection was complete, the needle was withdrawn and the injection site was rubbed in a posterior to anterior direction 10 times in order to avoid leakage from the site.

Toluene was mixed in various ratios with polyoxyethylated vegetable oil (Emulphor, EL610, Carney et al., 1977), to achieve an injection volume of 1.6 ml/kg of body weight. Dosages were 50, 100, 120, 250, 500 and 1000 mg/kg.

TABLE 1
MEANS, STANDARD DEVIATIONS, MINIMA AND MAXIMA OF WEIGHT
AND AGE OF FIVE GROUPS OF LONG EVANS RATS* USED IN THE SERIES OF STUDIES

Study	N	Weight				Age			
		$\bar{X}$	Std	Min	Max	$\bar{X}$	Std	Min	Max
I	44	374.9	38.6	307	462	-	-	-	-
II	96	361.1	33.9	291	500	77.4	1.9	72	83
III	24	356.3	25.4	327	435	78	0.0	78	78
IV (exploratory) includes Study III	54	390.6	35.9	327	467	82.2	3.8	78	86
IV (Confirmatory)	60	389.6	31.9	302	462	83.8	0.8	83	85

*Ten (10) Sprague Dawley rats initially included in Group I were later eliminated. Their data are not included in Table I.

Blood Sampling

Rats were injected in the morning and then housed in individual cages, in a lighted room without food or water until they were sacrificed. Rats were rendered unconscious by cervical dislocation and 3.5 ml of blood was drawn from the anterior vena cava as described in Benignus et al. (1981). Rats were sacrificed on 20 minute intervals after injection for up to 8 hours. Gas chromatography was used to analyze the blood samples (Benignus, et al., 1981).

Quantitative Methods

Previous data (Benignus, 1981, 1982) has shown that variance of blood levels is much larger for higher doses than for lower doses. Exploratory analyses in this study confirmed this. Consequently, all data were analyzed in log concentrations. The models of most interest are inherently nonlinear. Methods used for fitting inherently nonlinear equations (Gallant 1975b) were programmed in Statistical Analysis System (Helwig and Council, 1979). When parameter estimates for different nonlinear equations were to be compared, the technique of seemingly unrelated nonlinear regression (Gallant 1975a) was used.

SECTION 4

RESULTS

The results of the series of studies which were used to plan and execute the last two verification studies will be presented in order of their occurrence. The aim is to describe the steps and decisions made at each stage as well as to illustrate the variability of the data.

Study I (Exploratory)

The first group of exploratory rats (10 Sprague Dawley and 44 Long-Evans) were injected with 120 mg/kg of toluene. Only one dose level was used in order to discover the general time course of blood level. A wide variety of polynomial equations were tried (up to 6th order) in an effort to optimally fit the data for the time course of blood toluene. An equation for a 3-parameter analog of a single-compartment model as used in Benignus et al (1981) was also tried. The model was chosen here from statistical goodness of fit standards rather than from pharmacokinetics principles. The model equation is

$$\log C_i = \log \alpha + \log (1 - e^{-\beta t_i}) - \gamma t_i + \log \varepsilon_i \quad (1)$$

where C_i = the blood toluene concentration of subject i , t_i = the time after injection, and ε_i is an unknown random error associated with each subject. Here α is a parameter controlling the maximum blood level for fixed values of β and γ , β is a parameter for the rise rate and γ is a parameter for the fall rate. Throughout, the term "log" indicates natural (Napierian or base e) logarithms.

The squared correlation (R^2) between predictions made by estimating from equation (1) and the data was $R^2 = .52$, while a quartic polynomial model produced an $R^2 = .50$. Quintic and sextic models clearly were overparameterized. Since the single-compartment analog model (1) fit as well or better than

a more complex quartic model, and since it has a theoretical basis, the model (1) was chosen. Figure 1 shows the line of best fit using equation (1), its confidence bands and the observed data. Table 2 gives the values of the estimated parameters for equation (1), their estimated asymptotic standard errors and their asymptotic 95% confidence limits. Note that the confidence band refers to the line of best fit, i.e. the domain in which the line of best fit will be found. The confidence band does not refer to the individual observations. The specific equation for the prediction line shown in Figure 1 is

$$\log C_i = \log 6.66 + \log (1 - e^{-0.0214t_i}) - .00307t_i \quad (2)$$

Early in the course of this pilot work, it was incidentally noted that the 10 Sprague-Dawley rats had considerably lower blood levels, in all cases, than correspondingly exposed Long-Evans rats. No further work was done with Sprague-Dawley rats and their data were dropped from the above analyses.

Study II (Confirmatory)

At this point a confirmatory study was conducted to test equation (2) which had been developed in the exploratory study. It was hypothesized that the curve shape would be the same and only the maximum blood levels would vary as a function of dose level. Accordingly a new study was designed in which 96 rats were injected with either 50 or 500 mg/kg of toluene (n=48 per group). Tests were planned to compare all 3 parameters of the model for the 2 dose levels.

Equation (2) was independently fitted to the data from the 2 groups. Table 3 shows the estimates of the parameters, their estimated asymptotic standard errors and their asymptotic 95% confidence limits separately for each dose level.

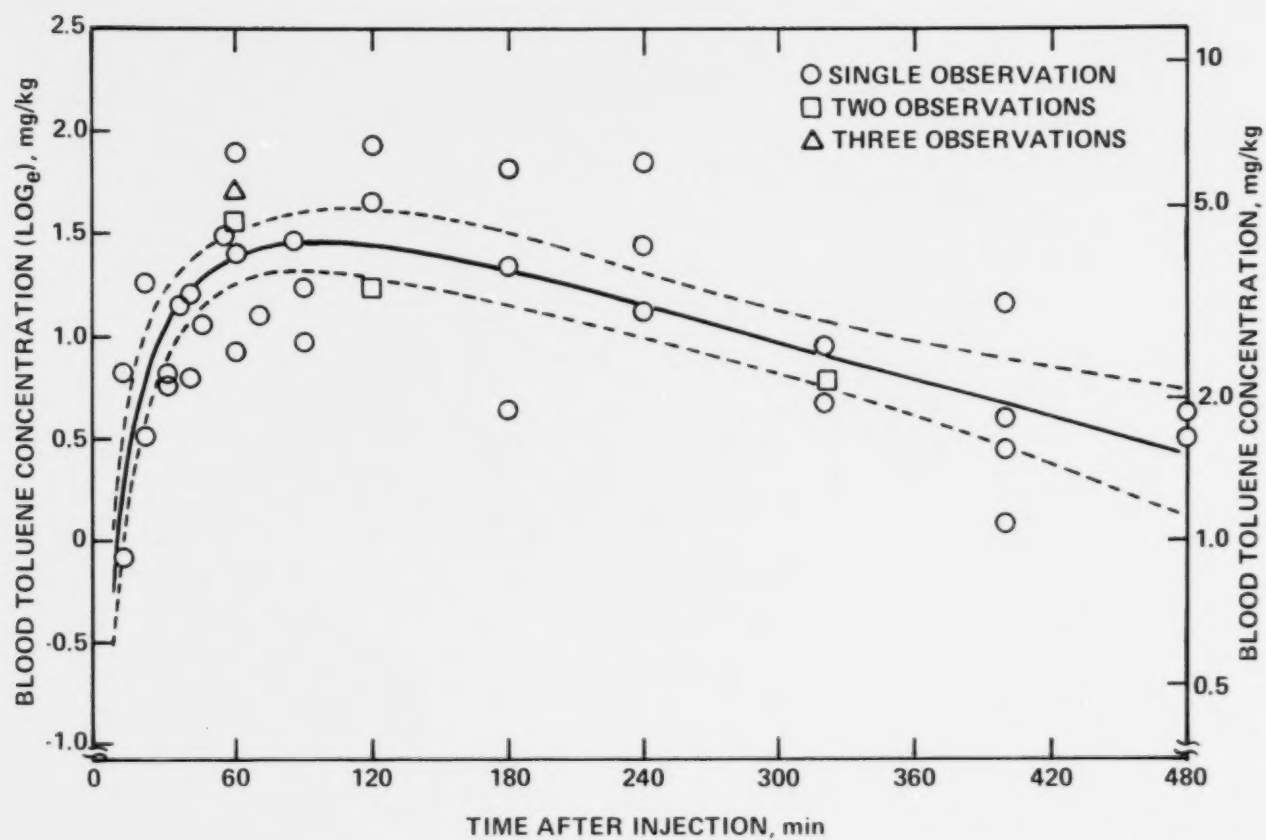


Figure 1.

TABLE 2
VALUES OF THE PARAMETERS ESTIMATED FOR EQUATION (1)
WITH STANDARD ERRORS AND 95% CONFIDENCE LIMITS
STUDY I

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95% Confidence Limits	
			Lower	Upper
α	6.66	1.50	3.63	9.69
β	.0214	.00735	.00635	.0365
γ	.00307	.000714	.00163	.00451

TABLE 3

VALUES OF THE PARAMETERS ESTIMATED FOR EQUATION (1) FOR 50 AND 500 MG/KG

DOSAGE, THEIR STANDARD ERROR AND THEIR 95% CONFIDENCE LIMITS

STUDY II

Dose	Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95% Confidence Limits	
				Lower	Upper
50	α	1.49	.284	.921	2.06
	β	.0342	.0167	.000437	.0679
	γ	.00179	.000603	.000571	.000300
500	α	8.96	1.75	5.45	12.5
	β	.0267	.0113	.00395	.0494
	γ	0.00	.000599	-.00121	.00121

Using approximate t tests (Gallant 1975b) it was concluded that rise rate, β , was not different for the two dose levels ($p > .6$). In contrast, α , which describes maximum level, differed ($p < 10^{-4}$) such that the higher dose had a larger α . Furthermore, γ , the fall rate, differed ($p < .004$) such that the higher dose had a smaller (slower) fall rate. Figure 2 shows the two lines of best fit, confidence bands and observed data.

Study III (Exploratory)

Study II results indicated that the 3-parameter model fit the data for each dose separately. However, both α and γ varied as a function of dose. If equation (1) were to be used, different parameter values would need to be estimated for each dose, a highly inconvenient procedure. Consequently, equation (1) needed to be generalized to allow for varying dose levels. This was done for statistical modeling rather than pharmacokinetic reasons. To help do this, a new group of rats, injected with 250 mg/kg of toluene was studied. The data from this new group was then merged with the 50 and 500 mg/kg data to form a 3-group data base. This 3-group data base was then used as exploratory data to devise a more general model.

Equation (1) was expanded to include dose-dependent increments for α , β and γ . The new equation has 6 parameters

$$\begin{aligned} \log C_i = & [\log \alpha_0 + (\log \alpha_1)(\log D_i)] \\ & + \log \{[1 - \exp[-t_i(\beta_0 + \beta_1 \log D_i)]] \\ & - t_i[\gamma_0 + \gamma_1(\log D_i)] + \log \epsilon_i \end{aligned} \quad (3)$$

where C_i = the blood toluene level for rat i, D_i is the dose level in mg/kg for rat i ($50 \leq D_i \leq 1000$), and t_i is time after injection in min, ($20 \leq t_i \leq 480$). The 6 empirical parameters are divided into the α , β and γ

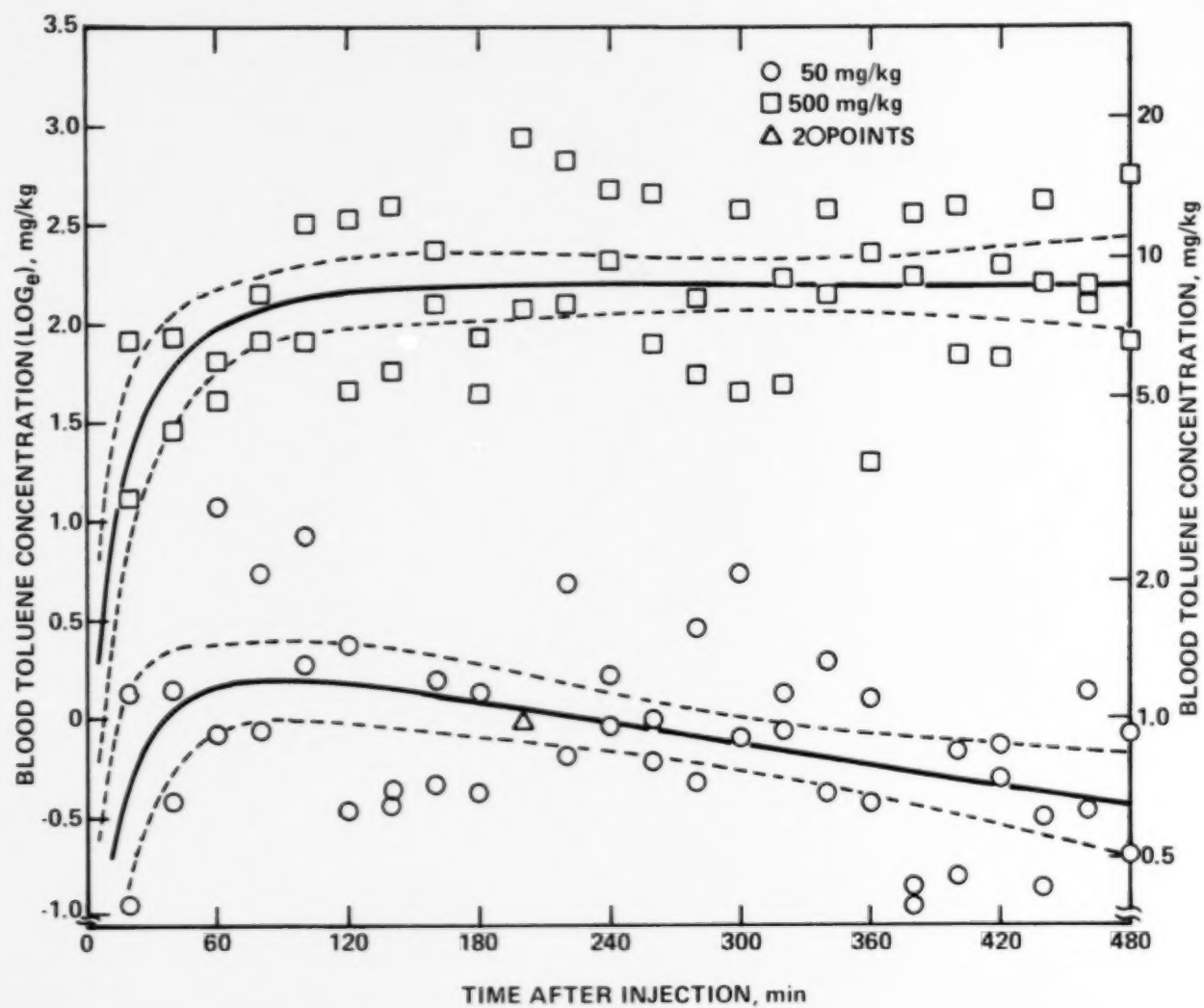


Figure 2.

groups, where each group corresponds to a single parameter in equation (1). Parameters subscripted with 0 are used to fit the "main effects" and parameters subscripted with 1 are used to account for the differential effects of dose level. The equation was fitted to the combined 3-dose data (Study II rats injected with 50 and 500 mg/kg and the new group injected with 250 mg/kg).

Attempts to fit equation (3) to the 3-dose data were unsatisfactory. Examination of raw data revealed that the data from the new 250 mg/kg group almost overlaid the previously collected 500 mg/kg data. Apparently some factor in the new group was different. It was not clear whether this difference might be due to a "batch" factor, time of year or procedural variations. Here "batch" refers to a group of rats received from a supplier in an intact group or at a single point in time.

Study IV (Exploratory and Confirmatory)

At this point it was decided to eliminate the possible batch factor by collecting exploratory and confirmatory data from rats in the same batch. This implied exposing all rats in one experiment and using only exploratory data to devise a model. The model could then be tested with the confirmatory data. Experimenters were to remain ignorant of the confirmatory data until exploratory data were completely analyzed.

Consequently, the following steps were planned. First 90 additional rats from the same batch as Study III animals were randomly assigned to either the exploratory group ($n = 30$), or the confirmatory group ($n = 60$). The exploratory subjects were randomly assigned to a 100 mg/kg dose ($n = 18$), a 250 mg/kg dose ($n = 6$) or a 1000 mg/kg dose ($n = 6$). Data from the 250 mg/kg

group in Study III were included as part of the pilot data. For the confirmatory study 12 animals were randomly assigned to each level: 50, 100, 250, 500 and 1000 mg/kg. Technical problems forced the elimination of 3 rats. Models were to be fitted to the 3 level exploratory data. After all analysis was complete and a final model chosen, it was to be tested with the 5 level confirmatory data.

Exploratory. Figure 3 shows the prediction lines from equation (3) as fitted to 100, 250 and 1000 mg/kg exploratory data. All observed data are also plotted. Confidence bands are included only for 100 and 250 mg/kg groups since too few animals were observed at 1000 mg/kg. The squared correlation of predicted with observed data was $R^2 = .81$. Table 4 shows the estimated values for the parameters of equation (3), their estimated asymptotic standard errors and their 95% confidence limits. The quality of residuals (trends, means and variances for each dose) indicated that the model had been adequately specified, while the high R^2 indicated that the model fit well.

Confirmatory. Equation (3), using all 6 parameters could account for the time trends in blood toluene level. However, the parameters may not have all been needed. By testing each parameter, the model, (3), could be reduced to its most parsimonious form.

The first step of the confirmatory analysis was to fit the six parameter model to the confirmatory data. Table 5 gives parameter estimates, estimated asymptotic standard errors and associated asymptotic 95% confidence limits. Note that all parameters have estimated confidence intervals which cover zero. Approximate F-tests (Gallant, 1975b) are also reported which test whether each parameter significantly helps to reduce the residual sums of squares. Only γ_0 passed this test. Residual analysis, including

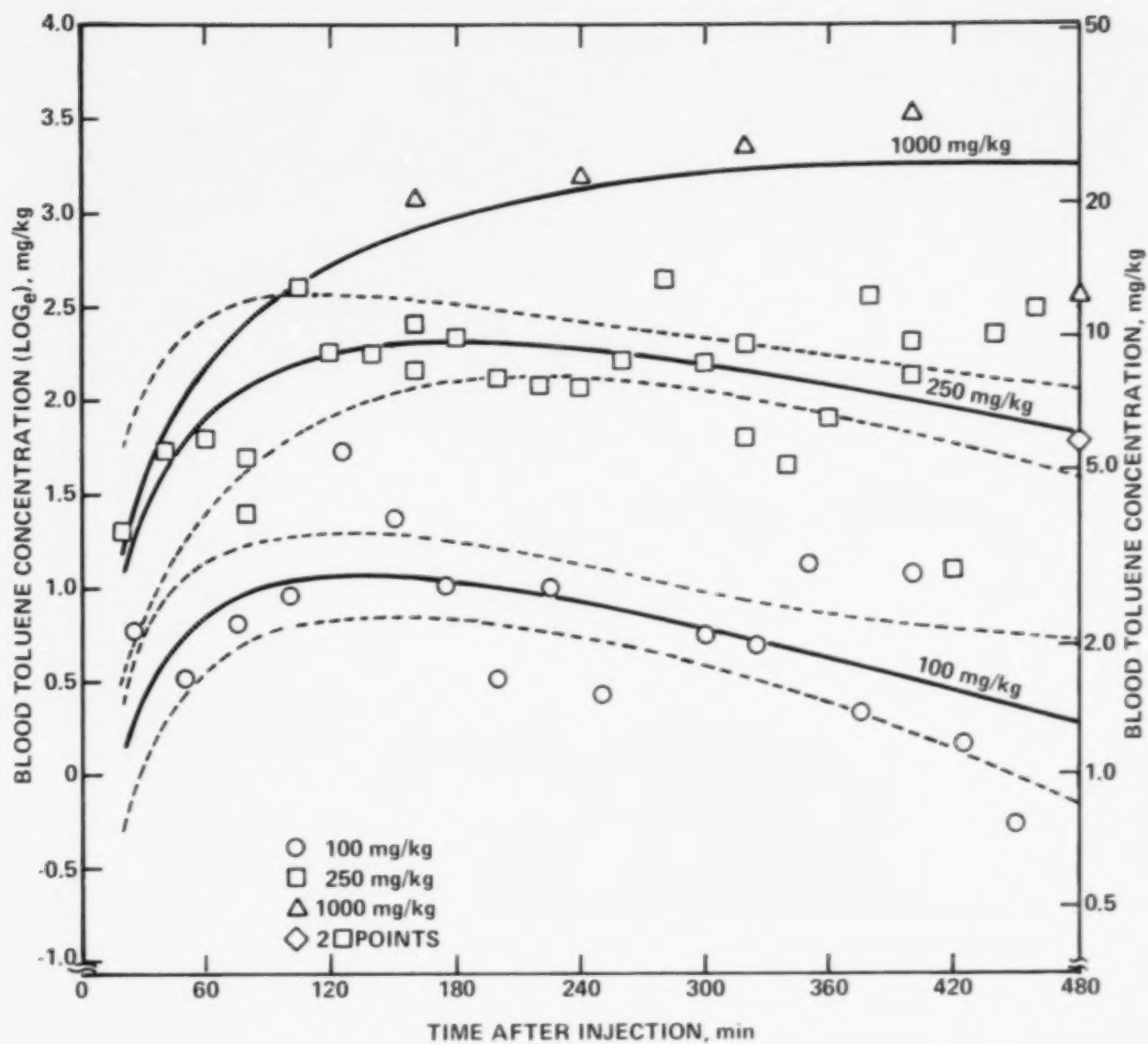


Figure 3.

TABLE 4
VALUES OF PARAMETERS ESTIMATED FOR EQUATION (3), THEIR STANDARD
ERRORS AND THEIR 95% CONFIDENCE LIMITS FOR EXPLORATORY STUDY IV
100, 250 AND 1000 MG/KG INJECTED GROUPS

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95% Confidence Limits	
			Lower	Upper
α_0	.0055	.0064	-.0074	.0184
α_1	4.4500	.9240	2.5800	6.3100
β_0	.0349	.0251	-.0156	.0854
β_1	.0049	.0045	-.0042	.0140
γ_0	-.00044	.00072	-.0019	.0010
γ_1	-.0049	.0036	-.0121	.0023

TABLE 5

VALUES OF PARAMETER ESTIMATES FOR EQUATION (3), STANDARD ERRORS, 95% CONFIDENCE LIMITS AND ASYMPTOTIC F-TESTS FOR THE HYPOTHESES THAT EACH PARAMETER DOES NOT ACCOUNT FOR A SIGNIFICANT AMOUNT OF THE RESIDUAL SUM-OF-SQUARES

STUDY IV CONFIRMATORY

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95% Confidence Limits		Asymptotic F	df num.	df den.	p<
			Lower	Upper				
α_0	2.85	11.2	-19.6	25.3	.28	1	54	.60
α_1	1.37	.917	-.473	3.20	2.06	1	54	.16
β_0	.00483	.0324	-.0602	.0698	.19	1	54	.67
β_1	.000471	.00578	-.0111	.0121	.03	1	54	.87
γ_0	.00941	.00792	-.00648	.0253	6.38	1	54	.02
γ_1	-.00127	.00138	-.00404	.00149	3.52	1	54	.07

evaluation of mean and variances separately for each dose indicated that the model was sufficient. R^2 was .77. Taken together, the results indicated that the model was over-parameterized, i.e., that fewer than six were needed. Furthermore, this indicated that some sub-model should fit essentially as well. Figure 4 shows the prediction lines from this model for 50, 100, 250, 500 and 1000 mg/kg dose levels. Figures 5, 6, 7, 8 and 9 show the same 5 prediction lines, associated asymptotic confidence bands and the corresponding observed data from the confirmatory data base.

The second step of the confirmatory analysis was to use the parameters estimated from the exploratory analysis to predict the observed confirmatory data. It was immediately noted that the data from the 100, 250 and 1000 mg/kg groups fit the predictions, but the data from the 50 and 500 mg/kg groups fit poorly. These data were therefore not plotted. Furthermore, data for groups for which corresponding groups were in the pilot study were predicted well. In contrast, data for groups without corresponding groups in the pilot study were predicted poorly. For the latter groups the prediction lines were unreasonable. An important example is that the predicted level for the 500 mg/kg group exceeded the predicted level for the 1000 mg/kg group. Such behavior again indicated that the model was overparameterized. Study IV (Reanalyzed)

It was decided to pool all of the data from the last group of 111 rats, from both the exploratory and confirmatory animals and use these data to estimate the 6 parameters of equation (3). In order to avoid inferential errors associated with stepwise testing of model parameters, a double cross-validation design was used (Mosier, 1951). The groups of rats

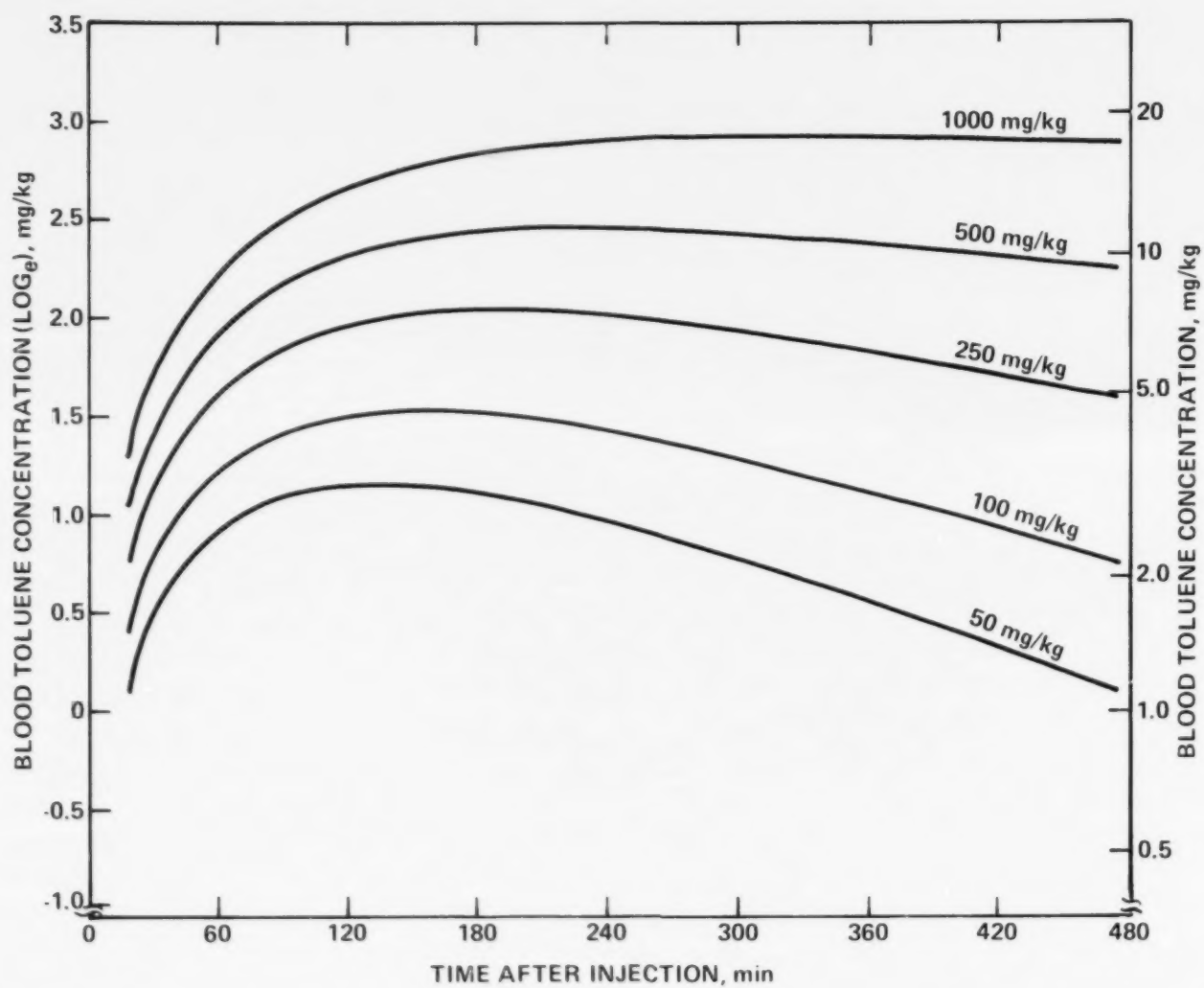


Figure 4.

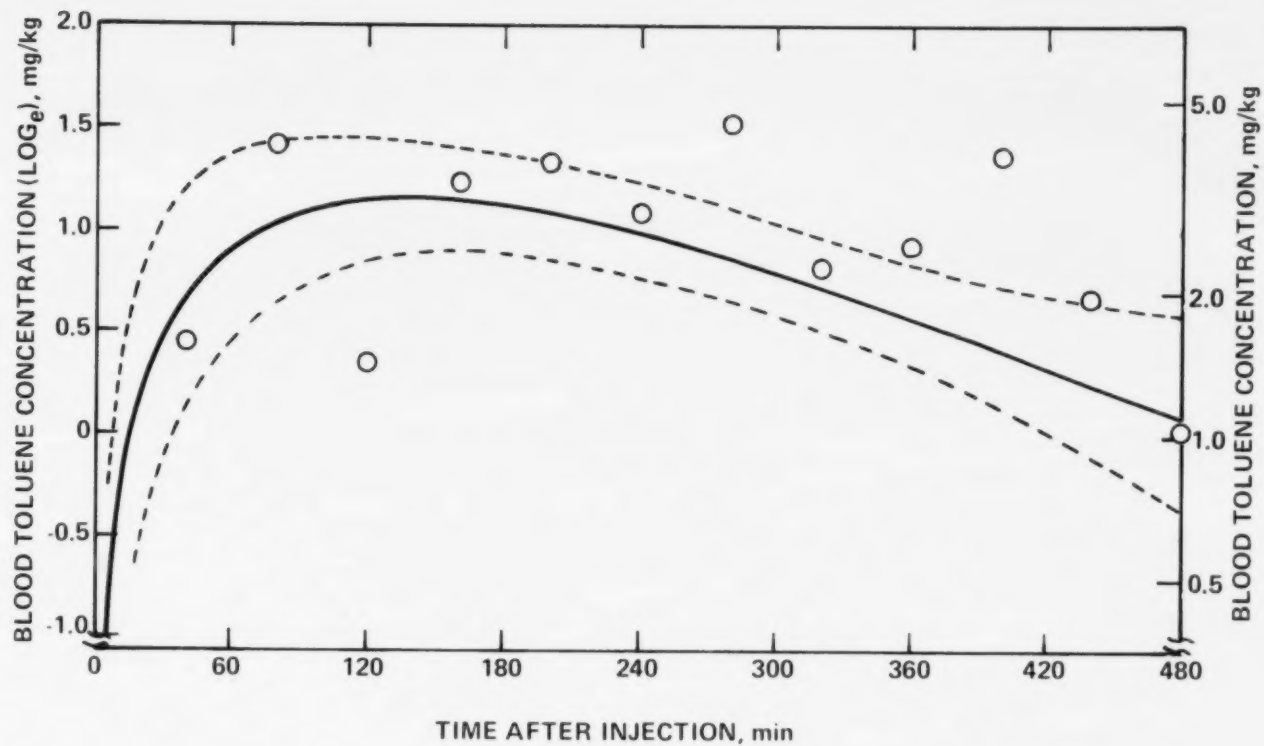


Figure 5.

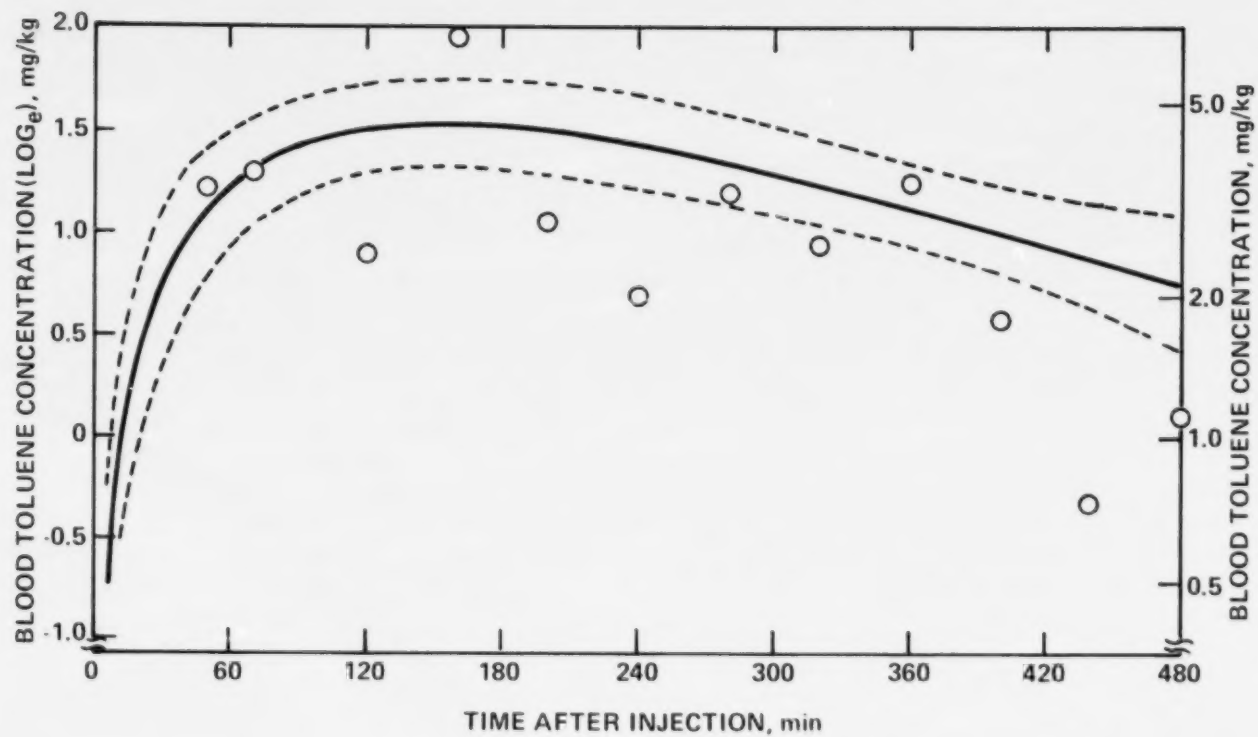


Figure 6.

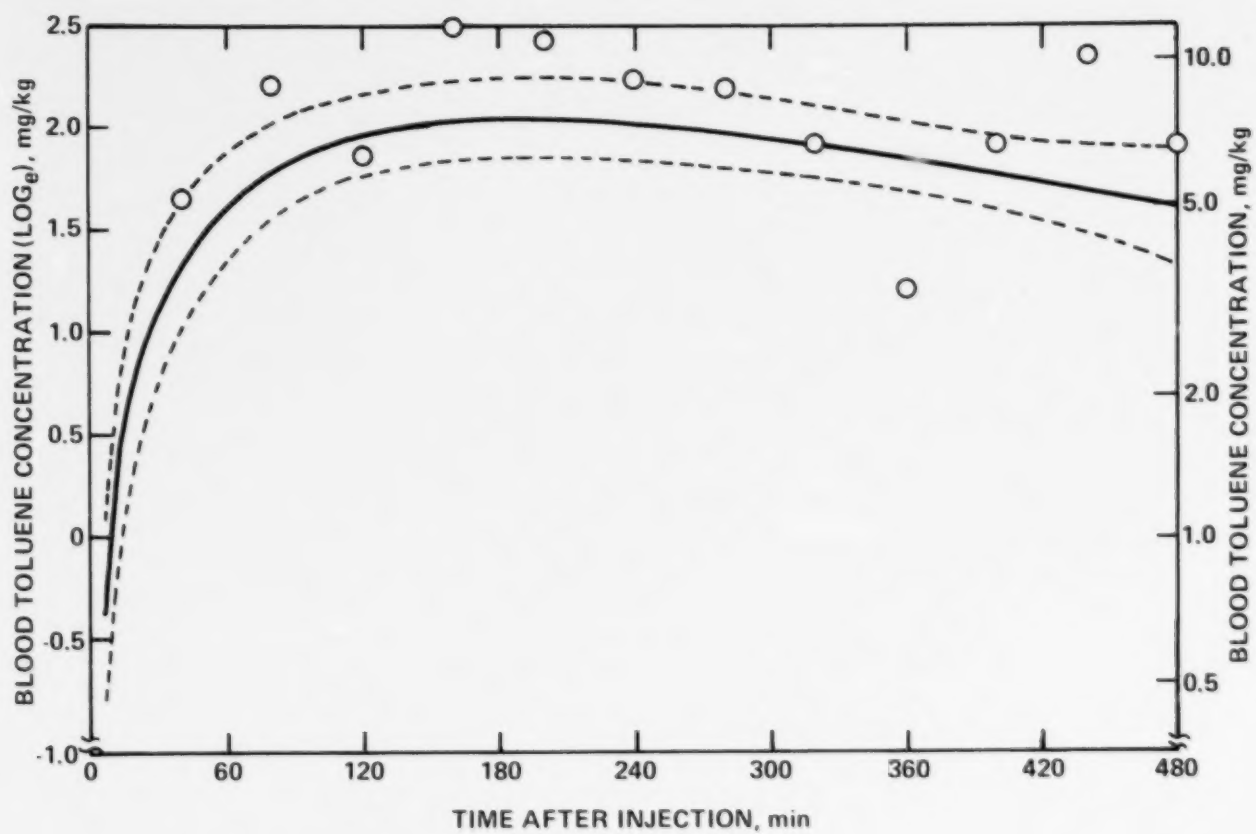


Figure 7.

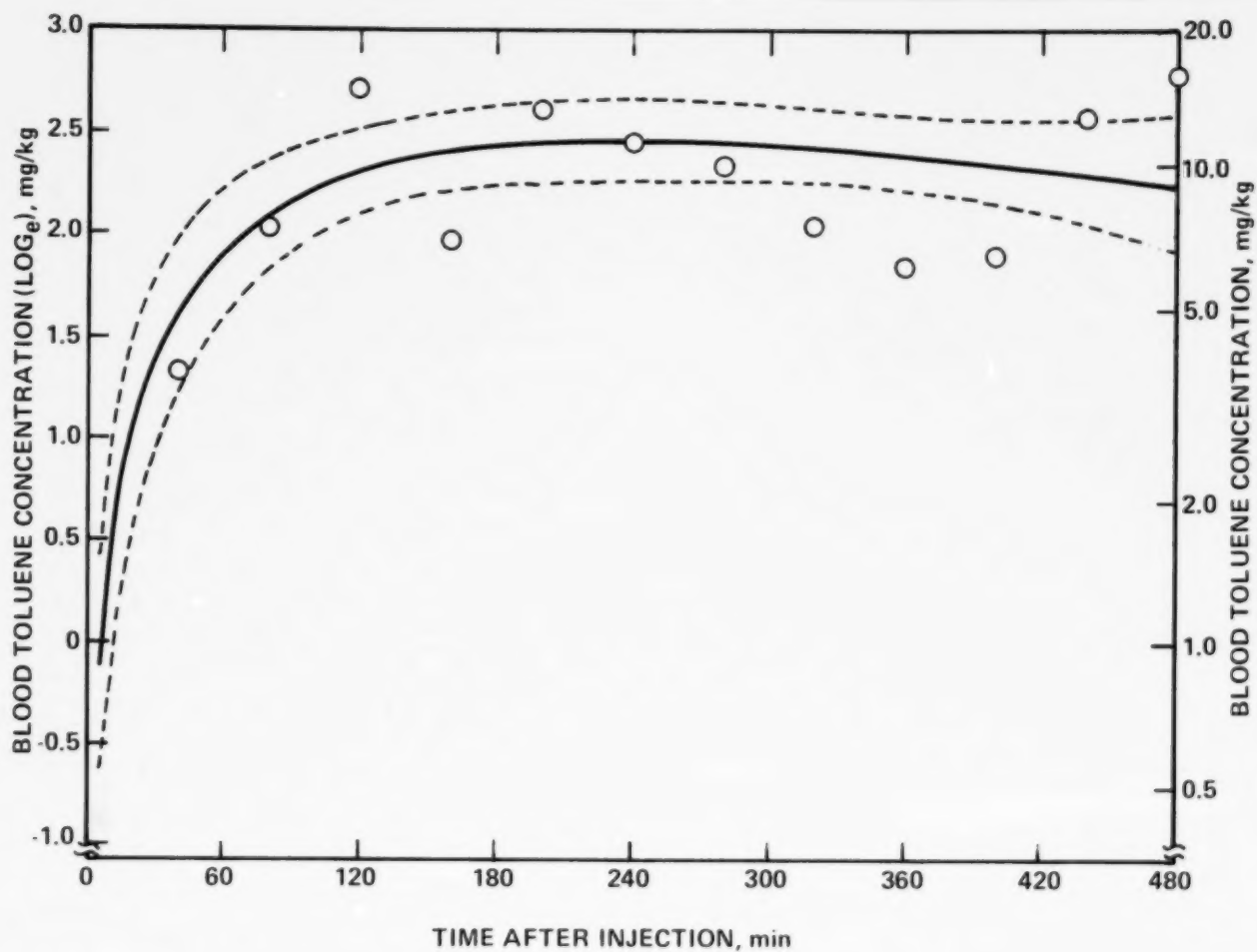


Figure 8.

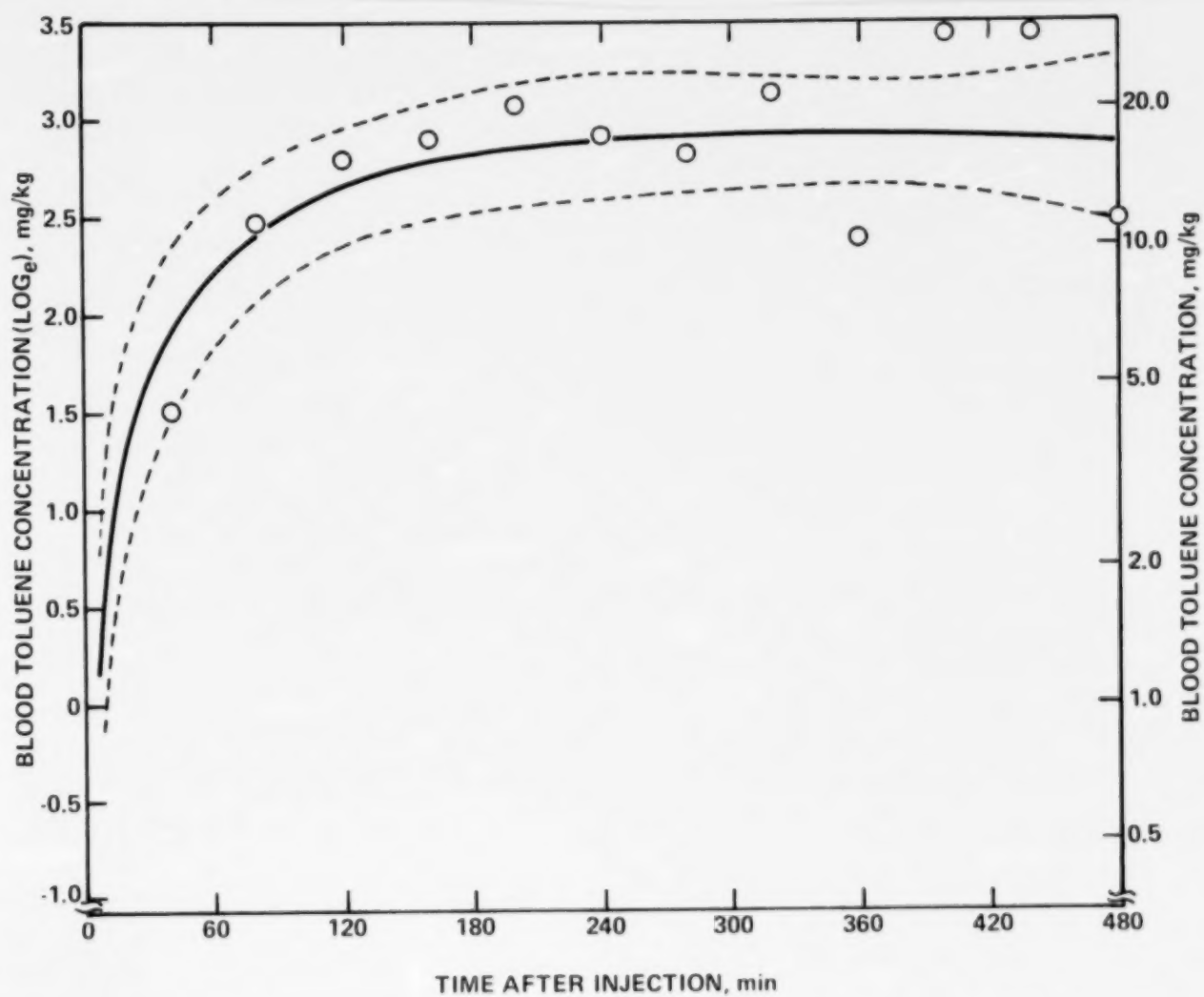


Figure 9.

were divided into two approximately equal halves by random sampling within dose level. A model was fitted to each half of the data, parameters tested and the best submodel was chosen. After each half had been fitted and reduced, the results from the two fitting procedures were compared to each other. The comparisons were performed by correlating the predictions using parameter estimates from one half with the observed data from the other half. Such a correlation is often called the "cross validation" correlation.

Table 6 shows the number of subjects in each of the dose-level groups as constructed from the combined groups. Each dose-level group was randomly split into halves A and B. Table 7 shows the results of fitting models to the two halves of the data. The first two rows give results from the full 6-parameter model and the second two rows are the results of a reduced model in which only 4 parameters were used (β_1 and α_0 having been eliminated).

This gives model (4),

$$\begin{aligned} \log C_i &= (\log \alpha_1)(\log D_i) \\ &+ \log [1 - \exp(-\beta_0 t_i)] \\ &- t_i(\gamma_0 + \gamma_1 \log d_i) + \log e_i \end{aligned} \quad (4)$$

Earlier results led to the hypothesis that β_1 and α_0 would not be needed. The last two columns of Table 7 show tests of the significance of the difference between the goodness of fit for the 6-parameter model and the corresponding reduced 4-parameter model for both halves of the data. From Table 7 it may be seen that α_0 and β_1 could be eliminated to produce a 4-parameter model which did not predict significantly more poorly than the 6-parameter model ($p > .61$ for half A and $p > .39$ for half B). Correlation of predictions

TABLE 6
NUMBER OF RATS IN EACH DOSE LEVEL OF A SPLIT-HALF ANALYSIS
(RANDOM SELECTION WITHIN EACH DOSE LEVEL)
STUDY IV REANALYZED

	Dose Level (mg/kg)					
	50	100	250	500	1000	Total
Sample A	6	15	20	6	9	56
Sample B	6	14	21	6	8	55

TABLE 7
TESTS COMPARING 6- AND 4- PARAMETER MODELS IN EACH SPLIT SAMPLE
STUDY IV REANALYZED

Sample	Parameters In Model	N	df _{model}	df _{err}	SS _{model}	SS _{total}	R(Y,Y)	$\tilde{F}$	p<
A	$\alpha_0 \alpha_1 \beta_0 \beta_1 \gamma_0 \gamma_1$	56	6	50	12.7	46.4	.852		
A	$\alpha_1 \beta_0 \gamma_0 \gamma_1$	56	4	52	13.0	46.4	.849	.51	.61
B	$\alpha_0 \alpha_1 \beta_0 \beta_1 \gamma_0 \gamma_1$	55	6	49	8.4	36.8	.878		
B	$\alpha_1 \beta_0 \gamma_0 \gamma_1$	55	4	51	8.8	36.8	.878	.97	.39

with actual observations revealed that the 4-parameter model accounted for only a trivial amount less variance than did the 6-parameter model.

Table 8 shows the cross-validation correlations. From these results it appears that the 4-parameter model, with parameters estimated from one half of the data, predicted the complementary half's data nearly as well as the 4-parameter model with parameters estimated from the same half. This leads to the conclusion that a reliable process of model selection and fitting occurred, and that capitalization on chance was minimized. Hence, it is reasonable to expect that these results would be replicated if a similar study were conducted in the future.

After it was decided that a stable model had been fitted, all observations from the two halves were combined and the fitting procedure was repeated to assure the most accurate estimates of parameters. Figure 10 shows the prediction from the model which was fitted to all 111 observations. Also shown on Figure 10 are estimated times during which the blood toluene level reaches and remains within 90% of its estimated maximum ppm value. Table 9 gives the 90%-of-maximum times shown in figure 10. The procedure was to first find the time of-maximum estimated ppm blood toluene level. The maximum level, MAX, (given in Table 9), was used to estimate the maximum blood toluene level in ppm (e^{max} of Table 9). Next 90% of the estimated maximum blood toluene level in ppm was computed. Finally, the two times, one before and one after the time of the maximum, were found which gave this 90% level as a prediction.

The variation of blood toluene level from 20-480 min. after injection is predicted for 50-1000 mg/kg of SC injected toluene. The model should be

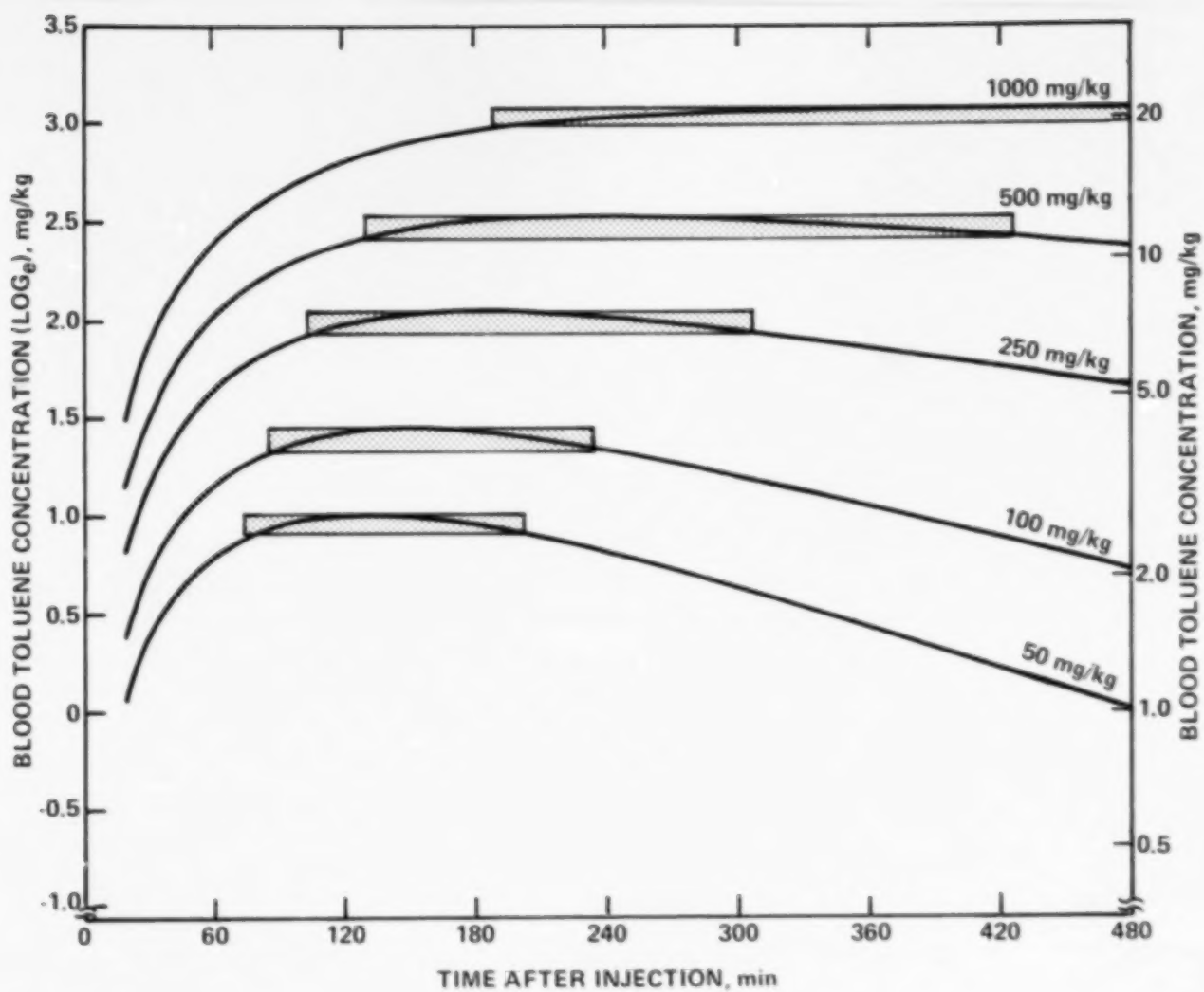


Figure 10.

TABLE 8
 DOUBLE CROSS-VALIDATION RESULTS
 (ENTRIES IN THE CELLS ARE CORRELATION COEFFICIENTS FOR THE PREDICTION
 FROM THE 4-PARAMETER MODELS WITH OBSERVED DATA)
 STUDY IV REANALYZED

		Half Of Observations	
		A	B
Half Of Data With Which Parameters Were Estimated	A	.849	.869
	B	.848	.873

TABLE 9
COMPUTATION OF ESTIMATES OF PERIODS WHEN BLOOD TOLUENE LEVELS ARE AT
90% OF MAXIMUM FOR EACH DOSE LEVEL
STUDY IV REANALYZED

Dose	Max Log (Toluene)	$e^{\max}$	$.9e^{\max}$	$t_{1, 90}$	$t_{2, 90}$
50	1.01	2.75	2.47	75	204
100	1.44	4.21	3.79	85	236
250	2.03	7.64	6.88	104	308
500	2.52	12.44	11.19	130	427
1000	3.08	21.86	19.68	190	>480

applicable throughout this range. The specific equation for this family of graphs, using notation established in equation (4) is:

$$\begin{aligned} \log C_i = & (\log 1.58)(\log D_i) \\ & + \log [1 - \exp(.0106 t_i)] \\ & - t_i(.00838 - .0012 \log D_i) \end{aligned} \quad (5)$$

Table 10 gives the estimated parameter values in equation (5) along with their 95% asymptotic estimated confidence limits. Note that none of the confidence intervals now covers zero.

In order to depict the variability about the predicted lines from the model, Figures 11 and 12 were constructed. Figure 11 shows the predicted curves for 50, 250, and 1000 mg/kg along with the observed data points. Figure 12 shows the 100 and 500 mg/kg predicted curves and the observed data. In both cases the asymptotic 95% confidence bands about the regression lines are included. Residual analysis of mean, variance, and trend for each dose separately, indicated that the model was appropriate for the data. Furthermore, the R^2 was also high, $R^2 = .74$.

In order to compare the variability of the results for the injection method to the variability observed in a previous study using inhalation (Benignus, *et al.*, 1981), an F test was used. Table 11 shows the results. The variability in the injection method is marginally larger but not significantly so. The functional importance of a difference so small (a ratio of 1:1.4) is debatable. This compares the methods strictly from a statistical perspective. No comparison of pharmacokinetics is intended.

TABLE 10
VALUES OF PARAMETERS AND CONFIDENCE LIMITS IN 4-PARAMETER
MODEL ESTIMATED FROM 111 RATS
STUDY IV REANALYZED

Parameter	Estimate	95% Confidence Limits	
		Lower	Upper
α_1	1.58	1.40	1.75
β_0	.0106	.0018	.0195
γ_0	.00838	.00662	.01014
γ_1	-.00120	-.00023	-.00744

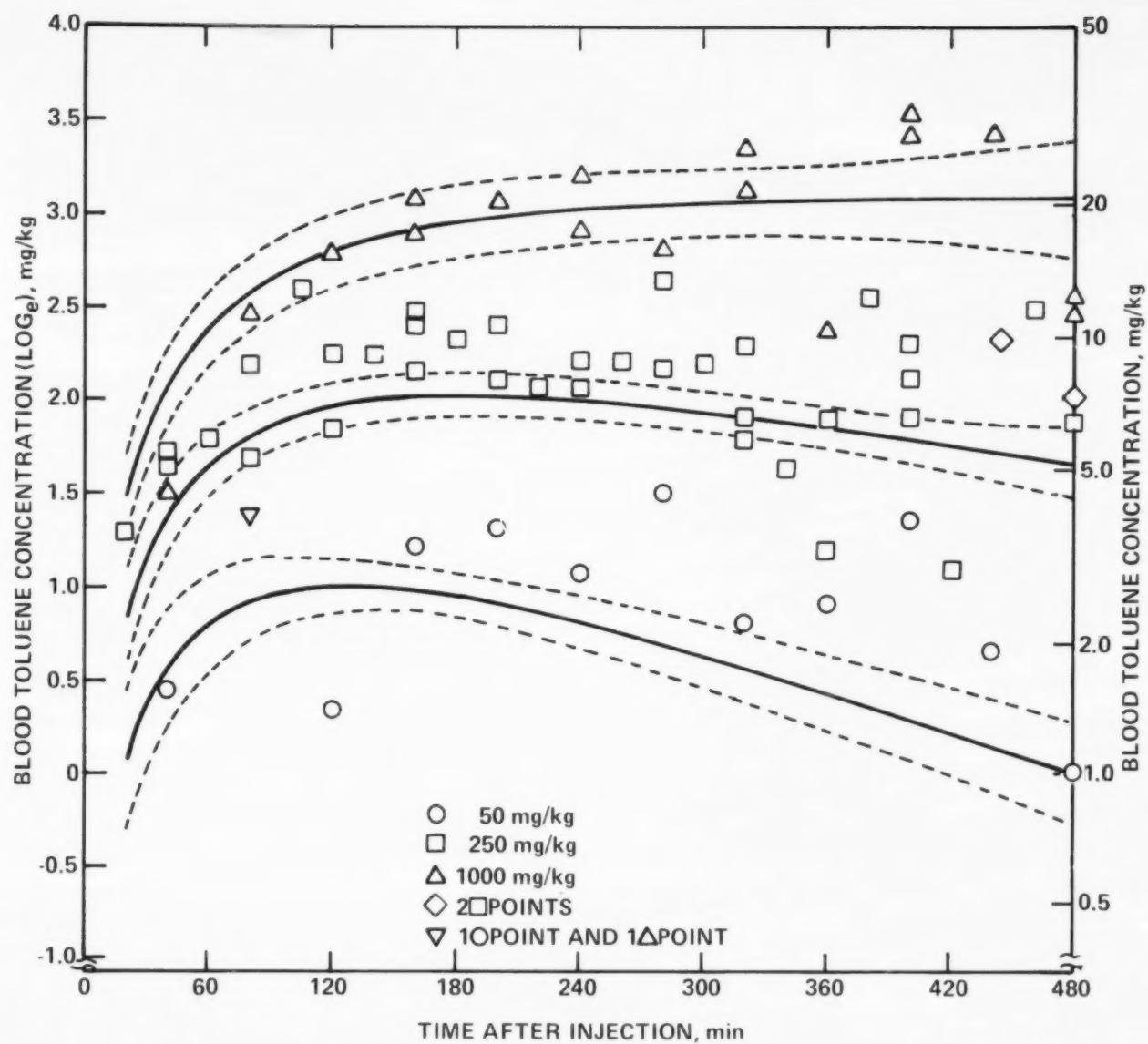


Figure 11.

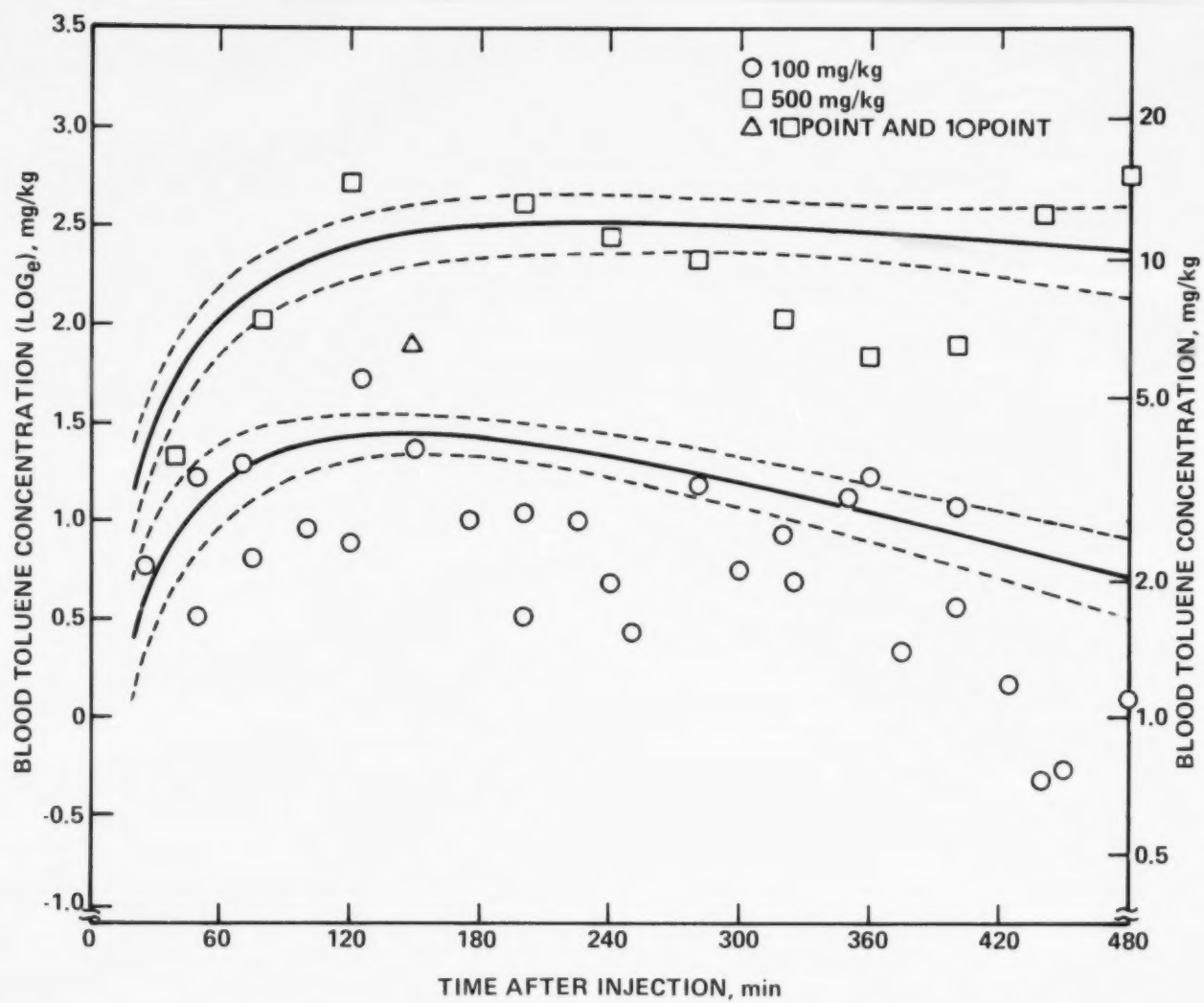


Figure 12.

TABLE 11
COMPARISON OF THE VARIABILITY OF INJECTION WITH INHALATION METHODS
STUDY IV VS BENIGNUS ET. AL., 1981

Route	N	MSE	Error df	Approx. F	P
Injection	111	.20378	107	1.40897	.05617
Inhalation	80	.14463	77		

SECTION 5

DISCUSSION

The Model

A model, (5), has been devised to describe the time course of log toluene level in blood after SC injection of toluene in an Emulphor vehicle. The goal was to be able to predict received dose in subsequent toxicologic studies. The parameters of the model are not independent, and each parameter affects the entire curve. Following are interpretations of the parameters and how the parameters and their interactions affect different parts of the curve. The model was chosen for statistical goodness of fit reasons rather than from pharmacokinetic principles.

Log toluene level in blood at any point in time may be described as the sum of three terms. The first term,

$$\log 1.58 (\log D_i), \quad (6)$$

is an index of the maximum level of log toluene in blood as a function of only the dose level, D_i . The second term (which is added to the first),

$$+ \log [1 - \exp(-.0106t_i)], \quad (7)$$

describes the rising limb of the curve as a function of time only. This term does not, therefore, vary with dose level. The third term in equation (5) is

$$- [.00838 - .0012 (\log D_i)] t_i, \quad (8)$$

which describes the falling limb of the curve. Since the last term contains both time, t_i , and dose, D_i , the slope of the falling limb of the curve is determined by dose level.

Maximum levels. The first term implies that the maximum level which is reached by log toluene level in blood is strictly a function of dose level.

Figure 10 shows that, as expected, the toluene level in blood agrees with the dose level in an ordinal fashion. It is predicted that maximum possible log ppm levels are linear functions of log mg/kg dose level. This is consistent with results of Benignus et al. (1982), for inhalation methods.

The Rising Limb. Examination of the middle term of equation (5) reveals that the rising limb of the curve describing log toluene level in blood should be independent of dose level. Inspection of Figure 10 and Table 9, however, reveals that higher doses require longer times to reach 90% of asymptote. This apparent contradiction can be explained as follows. At any point in time the level of log toluene in blood is described by the model as the sum of the three terms (6, 7 and 8). The last term describes the falling limb. Note that the absolute value of the last term increases as a function of time. Thus the model implies that toluene in blood is being eliminated at all non-zero times. For this reason none of the curves reach their maximum possible value. Since the model predicts a more rapidly falling log toluene level for lower dose levels, the log toluene levels for the lower dose levels do not approach their maximum possible levels as closely as do higher dose levels. The empirical maximum is also earlier for lower dose levels because the falling-limb portion, (8), begins to affect the curve earlier. Thus, despite the independence of dose level of the rising limb of the model, the empirical maxima occur later for higher doses.

The Falling Limb. The model as described in equation (5) predicts that log toluene level in blood should decrease more rapidly for lower dosages (after maximum level has been reached). This is due to the negative sign of γ_1 in expression (8). Figure 10 shows that the curves behave as expected and log toluene level in blood falls much more rapidly for lower dose levels.

Interpretation of the Model. To the extent that the model has heuristic value, it may permit physical interpretation. While the following is clearly hypothetical, it may be instructive. The fact that the slope of the falling limb of the curve of toluene level in blood is an inverse function of dose level implies that either metabolic elimination varies as a function of dose or that the subcutaneous bolus of toluene is being exhausted. The latter interpretation is more reasonable. If all boluses had a constant size, within experimental error tolerance, and the rate of removal of toluene from the bolus were constant, then the lower dose boluses would be exhausted earlier. Thus it appears that the temporal characteristics of these curves, both the rising and falling limbs, are influenced by the exhaustion of toluene from the subcutaneous bolus.

Utility of the Method

The fact that larger doses require longer elimination time makes this method of toluene exposure somewhat troublesome. For some purposes this may be an advantage, however. For larger doses, there was no decline in toluene blood levels even 8 hours after injection was observed. Extrapolation from the model of equation (5) leads to the probably absurd

prediction that as much as 434 hours might be required before toluene blood levels decline to 10% of maximum after injection of 1000 mg/kg toluene. Since no data were collected beyond 8 hours, however, this extrapolation should be taken as a very crude estimate. Such a long elimination time may imply that daily injection would have cumulative effects of unknown magnitude. If high dose levels are employed, daily injections may not be feasible. Alternatively, it is reasonable to hypothesize that the bolus may not be exhausted until, for example, 10 hours. If true, then elimination could occur rapidly (perhaps in a few hours) thereafter.

Injection and inhalation differ with respect to repeated dosing. Two disadvantages of injection are 1) inability to provide high level short time exposures (rapid up/down cycle) and 2) possible mechanical problems of rat health with repeated injections. Within these limitations, it is still feasible to use repeated injections. Note that the model can still be used, with some additional work.

Groups which have been injected with different dosages differ in maximum levels of toluene in blood, also differ in the time required to reach 90% of maximum, the time required for toluene levels to decline and consequently, the time of exposure at near-100% levels. These temporal characteristics make SC injection a poor simulation of the normal route of exposure, inhalation. Despite the poor simulation of temporal characteristics, there is sufficient time, even for low doses, to perform many studies while toluene remains at 90% of maximum or above. If temporal characteristics are important, this method might act as a very poor exposure regimen. Otherwise SC injection could provide at least preliminary data about toluene's effects.

The range of doses and times were chosen to be of maximum interest in subsequent toxicologic studies. Since the model does fit well in the range observed, the primary purpose of predicting received dose was achieved. If the model is "taken too seriously" as a pharmacokinetics statement, it appears less useful.

Variability

As can be seen from Figures 11 and 12, empirical points scatter fairly widely about the predicted curves. Tests show, however, that this variability is not greatly larger than that produced by the inhalation method. It was not clear what the source of this variability could be. Variability in the site, size or depth of the injection, leakage from the site, variability in fatty tissues in the immediate injection site, and dose measurement error are all candidates. At present, this variability should be treated as inherent to the method.

Generality

Care should be used in extending these data beyond the circumstances of this study. Such variables as the kind of vehicle, the size of the injection, the activity cycle of the rats and the sex and strain of rats could all affect the outcomes.

Three basic ideas may well apply in fitting models for other compounds. First, a basic model for one dose may reasonably be found first, then generalized as a function of dose. Second, the log transform should be seriously entertained in modeling received dose, since errors tend to be proportional to the measurement. Finally, some form of split sample approach is mandatory to insure the replicability of any exploratory conclusions.

Conclusions

The time course of log toluene in blood following SC injection has been described. This should be useful in subsequent toxicologic studies, though of less use in understanding the pharmacokinetics. Maximum log blood levels are only determined by dose levels. Rise and fall rates of log toluene in blood are inverse functions of dose level. In general, the 4-parameters are not independent and each parameter affects the entire curve.

The SC injection technique appears to have definite but limited utility as a method of toluene exposure. It has the advantage of low expense and equipment requirements as well as simplicity. Its disadvantages reduce to poor temporal simulation of inhalation exposure for certain kinds of study.

Perhaps it would be most useful for preliminary work on the effects of toluene. It would usually seem to be the case, especially in behavioral work, that effects would have to be verified in inhalation exposures in order to generalize the results to the usual route of exposure.

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FIGURE CAPTIONS

- Figure 1. Line of best fit from the 3-parameter model (1), 95% confidence band and observed data.
- Figure 2. Lines of best fit from a 3-parameter model, 95% confidence bands and observed data for 50 and 500 mg/kg doses.
- Figure 3. Lines of best fit for a 6-parameter model, 95% confidence band and observed data for 100, 250 and 1000 mg/kg.
- Figure 4. Lines of best fit for a 6-parameter model for 50, 100, 250, 500 and 1000 mg/kg doses.
- Figure 5. Line of best fit for a 6-parameter model, 95% confidence band and observed data for 50 mg/kg dose.
- Figure 6. Line of best fit for a 6-parameter model, 95% confidence band and observed data for 100 mg/kg dose.
- Figure 7. Line of best fit for a 6-parameter model, 95% confidence band and observed data for 250 mg/kg dose.
- Figure 8. Line of best fit for a 6-parameter model, 95% confidence band and observed data for 500 mg/kg dose.
- Figure 9. Line of best fit for a 6-parameter model, 95% confidence band and observed data for 1000 mg/kg dose.
- Figure 10. Lines fo best fit for the 4-parameter model. The crosshatched bars show the times during which the toluene concentration in blood is estimated to be at least 90% of maximum (raw data) for each dose.
- Figure 11. Lines of best fit for the 4-parameter model, 95% confidence bands and observed data for 50, 250 and 1000 mg/kg doses.
- Figure 12. Lines of best fit for the 4-parameter model, 95% confidence band and observed data for 100 and 500 mg/kg doses.